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Oxygen Transfer in Fermentation

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The rate of biological processes in aerobic fermentation and waste water treatment is often limited by the rate of dissolution of oxygen from air bubbles into the liquid. A clear understanding of interfacial resistance to oxygen transfer is thus of importance. An oxygen sensing microprobe was used to detect a stagnant film and a penetrable zone near an air-water interface contaminated with surfactants. The technique, when used in clean water, can also measure the frequency of surface renewal. A linear relationship was found between the square root of the renewal rate $s^{0.5}$ and the interfacial mass transfer coefficient k_L, which is predicted by the Danckwerts theory.

SCOPE

Mass transfer is an important phenomenon in all fermentations which always involve several heterogeneous phases. Even the simplest system contains at least two phases: discrete microbiological cells and a continuous aqueous solution of nutrients. In oxygen requiring processes of aerobic fermentation and waste water treatment, a third phase of air bubbles is often introduced. Gas-liquid contacting in microbiological systems is complicated by the fact that oxygen consuming microbiological cells are often absorbed on surfaces of gas bubbles, which creates a nonuniform distribution between the interfacial zone and the rest of the reaction mixture. Mass transfer theories for ordinary gas-liquid contacting do not seem to hold in biochemical and biological processes as observed and discussed by Tsao (1972), Lee and Tsao (1972, 1973), and Tsao, Mukerjee and Lee (1972).

Based upon his results on carbon dioxide absorption by an aqueous buffer in the presence of an enzyme, carbonic anhydrase, Tsao (1972) postulated an interfacial mass transfer model involving two zones: a stagnant film heavy with absorbed microbiological cells and absorbed surfactants and a renewable zone penetrable by liquid elements due to turbulence in the liquid bulk.

In this paper, an experimental examination of the two zones was made by using a microprobe sensor at the surface of a protein solution. The microprobe was used to measure not only the thickness of the stagnant film but also the frequency of renewal in the penetration zone. The experimental technique was also employed to investigate the interfacial phenomenon in a clean water system and provided some valuable experimental evidence of surface renewal.

CONCLUSIONS AND SIGNIFICANCE

An oxygen microprobe is an inert material coated, long needle with an exposed sensing tip of about 2 μ long and 1 μ wide, which detects the local oxygen concentration and sends signals to a picoammeter for readout and recording. In this work an oxygen microprobe was attached to a micromanipulator which allows three-dimensional movements of the sensing tip with increments of 5 μ. When a microprobe was placed near an air-water interface, typical signals on a recorder chart are shown in Figure 3. The experimental apparatus given in Figure 2 is essentially a stirred, gas absorption cell. When the water was stirred with the agitator at different speeds (revolutions per minute marked in Figure 3), the signals (reflecting local oxygen concentration) were found to fluctuate at different frequencies. Each of the abrupt changes on the recorder curves was interpreted to reflect a renewal of the surface with a liquid element freshly brought to the interfacial zone by the agitator. By counting the fluctuation frequency on the recorder charts, it was possible to obtain a linear correlation between k_L and $s^{0.5}$, shown in Figure 4. This linear correlation agrees with Danckwerts's surface renewal equation

$$k_L = (D\,s)^{0.5}$$

and provides a direct experimental support to the Danckwerts theory.

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When surfactants, enzymes, and microbiological cells are added into water, surface absorption will create a nonuniform distribution of the surface active material between the region near the gas-liquid interface and the liquid bulk. This situation is common to all fermentations, and its resistance to oxygen transfer from the gas phase into the liquid is of fundamental importance to all processes involving oxygen consuming microorganisms. In this paper, a microprobe was also used to demonstrate, in the region near a surfactants laden interface, the existence of two transferring zones: a stagnant film and a penetration zone. The significance of Tsao's two-zone model in explaining the discrepancy among many oxygen transfer studies in the fermentation literature was discussed elsewhere (Tsao, Mukerjee, and Lee, 1972).

As shown in Figure 6, starting from its upper right corner, the sensing tip of the microprobe was lowered in discrete increments towards the liquid bulk by careful adjustments of the micromanipulator. The relative low fluctuation amplitude between interface and 0.24 (millimeters from the interface) on the recorded curve in Figure 6 indicates a stagnant film. The abrupt drop at 0.24 signals the complete penetration of the stagnant film by the downward movement of the microprobe. The initial violent fluctuation and the gradual decay in the amplitude from 0.24 on indicates the liquid penetration zone. At around 0.6, the fluctuation has died off almost completely, which indicates the microprobe reaching the liquid bulk where a uniform oxygen concentration should exist owing to mixing.

PREVIOUS WORK

Interfacial mass transfer is of fundamental importance in all aerobic fermentations which involve at least three phases: gas bubbles, nutrient solution, and discrete microbiological cells. Recently, liquid hydrocarbons are used as the carbon and energy source for producing single-cell protein. Hydrocarbons are often added into the fermentation vessels in yet another phase of dispersed droplets. Of current interest are also processes of microbial growth on cellulose containing solid wastes, on coal for sulfur removal, and on ores and ore tailings for mineral extraction. In such systems, materials and nutrients have to be transported from a solid phase into an aqueous solution and then into another solid phase, the cells. Frequently, at the same time, oxygen is to be transported from a bubble into a solution and then the cells to support growth. The rate of an overall fermentation process is often limited by the rate of a key mass transfer step. In 1950, two classical papers on oxygen transfer in fermentation by Hixon and Gaden and by Bartholomew, Karow, Sfat, and Wilhelm were published, an event which is generally referred as the beginning of Biochemical Engineering.

Since 1950, many additional publications have appeared in fermentation literature dealing with interfacial transfer of mass, particularly oxygen. However, even today there is yet no clear picture of the fundamental transfer mechanism in fermentation mixtures. Such mass transfer theories by Danckwerts and Higbie for ordinary gas-liquid systems do not seem to hold in microbiological processes, as discussed by Tsao (1972), Lee and Tsao (1972), and Tsao, Mukerjee, and Lee (1972).

Scale-up of a fermentation process is difficult and often done by rules of thumb rather than rational designs. The few available scale-up techniques are usually unreliable. The difficulty is at least partly due to the lack of a real understanding of the interfacial mass transfer process which is often a rate limiting factor of fermentation and thus should be a key criterion of scale-up.

A most commonly used technique for measuring oxygen transfer efficiency in a fermentor is based upon

$$SO_3^{--} + \frac{1}{2} O_2 \longrightarrow SO_4^{--} \qquad (1)$$

promoted by Co^{++} or Cu^{++} ions. In a sulfite solution, dissolved oxygen is continuously consumed in the presence of a suitable catalyst. The rate of disappearance of SO_3^{--}, often measured iodometrically, can be made to depend upon the rate of oxygen supply to the solution. In a fermentor, the term sulfite number reflecting the rate of

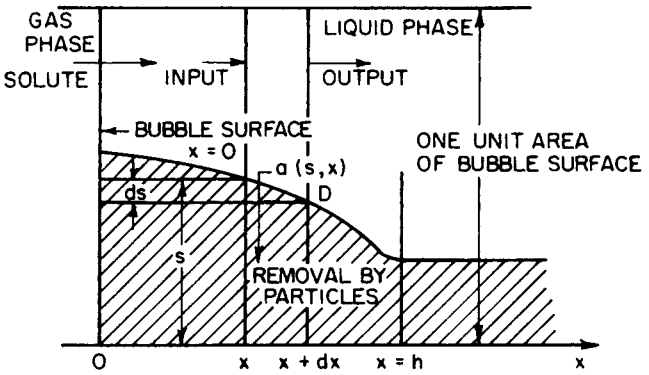


Fig. 1. Interfacial zone with nonuniformly distributed small particles.

SO_3^{--} consumption was thus often used as a measure of oxygen transfer efficiency. However, in sulfite oxidation, the interfacial oxygen transfer from air bubbles into the solution is not physical but involves a simultaneous chemical reaction. The difference between physical absorption and absorption with simultaneous chemical reactions is an important subject of two excellent books of Astarita (1967) and Danckwerts (1970). Oxygen absorption into a fermentation broth is not totally physical either but involves biochemical reactions in a different phase, the discrete microbiological cells. Furthermore, the cells tend to distribute nonuniformly owing to their surface properties. The failure in using the so-called sulfite number in scale-up of fermentation processes is at least partly due to the poor experimental simulation of a fermentation process by the sulfite oxidation reaction.

Using an isolated enzyme, glucose oxidase, Tsao (1968) demonstrated the effect of a simultaneous biochemical reaction on gas absorption and postulated a model incorporating the effect of a nonuniform distribution of microbial cells next to the interface. Figure 1 shows a diagram depicting a gas-liquid interface with microbial cells suspended in the liquid. A material balance of oxygen will yield

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} + D \frac{\partial c}{\partial x} \left[\frac{1}{1-s} \frac{\partial s}{\partial x} \right] - \frac{asR}{(1-s)} \qquad (2)$$

Solution of Equation (2) will depend upon, in addition to the boundary conditions, the cell concentration as a function of x, that is, $s = s(x)$, and also R which is a function of, among other things, the solute concentration c.

From the solution of Equation (2), the instantaneous rate of absorption can be obtained:

$$r = -D\left(\frac{\partial c}{\partial x}\right) \quad \text{at} \quad x = 0 \qquad (3)$$

A time average of r will give the rate of absorption that is measurable experimentally.

Some special solutions of Equation (2) with specific functions of s and R were reported by Tsao (1969, 1970) and Schierholz and Tsao (1971). The main thesis of Tsao and his co-workers is that, in fermentation, the discrete particles (the cells) will consume the transferring solute and thus can (but not necessarily always) enhance the interfacial mass transfer. This effect is further exaggerated by the tendency of microbiological cells crowding themselves at the interface due to surface absorption.

Experimental evidence of rate enhancement due to particles was observed in three bacterial cell suspensions as reported by Mukerjee (1973) and Tsao, Mukerjee, and Lee (1972). Interfacial transfer of iodine from toluene into an aqueous solution was also enhanced by the iodine reacting starch granules (Lee and Tsao, 1973). Air oxidations of glucose involving particles of platinum-on-carbon had also an enhanced oxygen absorption rate as reported by Lee and Tsao (1972). Solutions containing surface active proteins were also examined. Glucose oxidase was found to enhance oxygen absorption into a glucose solution (Lee and Tsao, 1972; Tsao, 1968), while carbonic anhydrase, an enzyme existing in animal blood, was found to enhance carbon dioxide absorption into a phosphate buffer (Tsao, 1972). Thus, the mechanism postulated originally for fermentation systems is also applicable to other systems involving reacting small particles and surface active reagents.

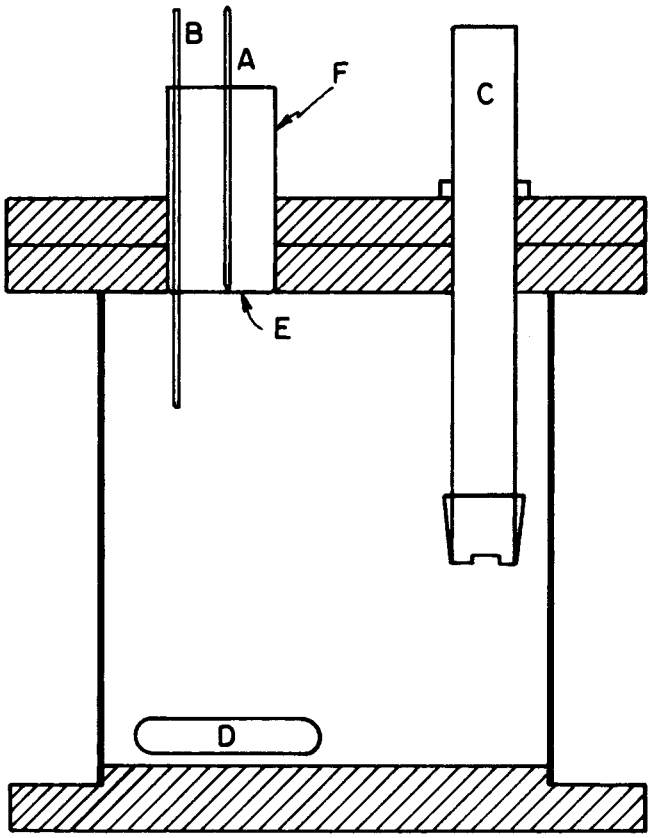
In defending his one parameter (s, the renewal rate) mass transfer theory, Danckwerts (1970) stated that many two- and multiple-parameter models can perhaps explain the fundamental phenomenon of interfacial mass transfer more fully, but they are certainly more difficult to use owing to the need of determining experimentally those extra parameters. Danckwerts also cited a number of examples in which the mass transfer rates predicted by his one-parameter theory are quite close to those by the more sophisticated models. Unfortunately, the one-parameter models do not seem to work in fermentation. According to Danckwerts

$$k_L = (D\,s)^{1/2} \qquad (4)$$

In the case of a fermentation broth, the gas-liquid interface is heavily coated with the microbial cells; it is thus difficult to visualize a mechanism involving a Danckwerts type of surface penetration. On physical grounds, one can speculate that, in order to describe the interface adequately, a parameter is needed to describe the hydrodynamics of the system, a second one to describe the cell density at the interface, and possibly even a third parameter to describe the kinetics of the uptake of the transferring solute by the cells. The detailed structure of the mass transferring interfaces is examined in this paper.

EXPERIMENTAL APPARATUS

Briefly, a microprobe is consisted of an inert material, coated, long needle with an exposed sensing tip of about 2μ long and 1μ wide, which detects the local oxygen concentration and sends signals to a picoammeter for readout and recording. With added adapters, the microprobes can be used to read also local concentrations of ionic species, carbon dioxide, and so on. In this work, a platinum, Model 721, oxygen microprobe, together with a Model 315 Ag-AgCl reference electrode, were connected to a Transidyne General Model 1200 picoammeter which was in turn connected to a Brush Mark 280 recorder capable of recording better than 60 Hz fluctuations.



A. Micro oxygen probe
B. Reference electrode
C. Bulk oxygen probe
D. Teflon magnetic stirring bar
E. Air-water interface
F. Tube on the cover

Fig. 2. A stirred absorption cell with a microprobe.

The absorption cell is shown in Figure 2 which is also attached with a Beckman Fieldlab oxygen probe for measuring the dissolved oxygen concentration in the liquid bulk. The absorption cell was designed to have the following special arrangements. It was stirred with a magnetic bar placed purposely off-center to avoid any vortex formation. The cell was filled with water or an aqueous solution to the very top. The only absorbing gas-liquid interface is that of E (in Figure 2) exposed to the gas in a tube F attached to the cell cover. These special arrangements were necessary precautions for preventing the surface area of the absorbing liquid from varying as the agitator speed was varied. The tube F was also enclosed so that the gas atmosphere in the tube was controlled.

In this work, the oxygen microprobe was fixed on a holder which was attached to a micromanipulator allowing three-dimensional movements of the sensing tip with increments of 5μ. The use of the micromanipulator made it relatively easy to place the sensing tip right at the gas-liquid interface. When the sensing tip was carefully lowered by adjusting the micromanipulator, an abrupt response on the picoammeter would signal touching of the tip with the interface.

RESULTS ON FREQUENCY OF SURFACE RENEWAL IN CLEAN WATER

An air-water pair was first used for absorption study. With all other conditions fixed, the liquid was agitated with the stirring bar at different rotating speeds measured

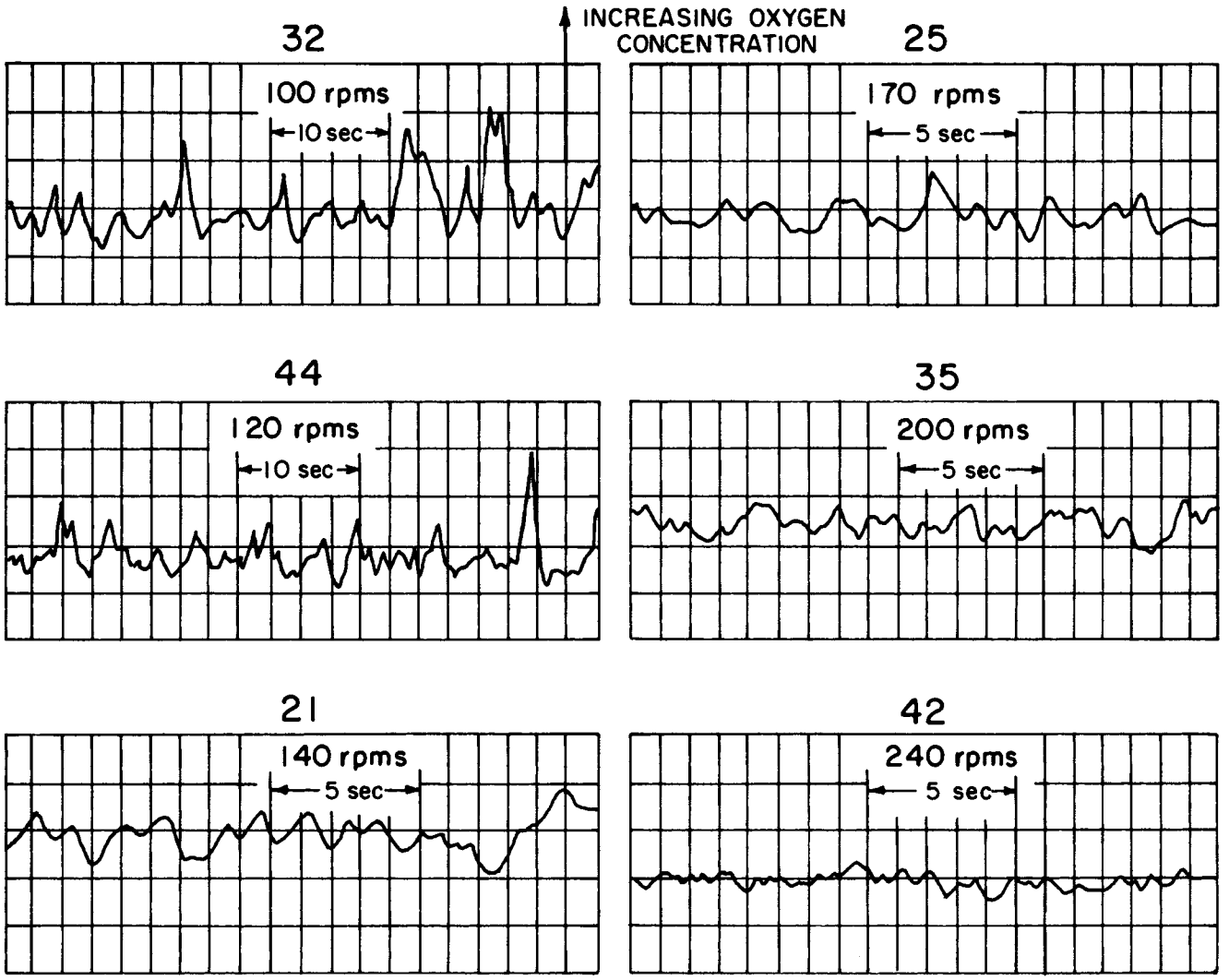


Fig. 3. Surface renewal rate at different agitator speeds detected by microprobe.

with a stroboscope. A typical recorder curve is shown in Figure 3. The frequency of fluctuation on the recorder curve is different at different stirrer revolutions per minute as marked. The abrupt changes on the recorder curves were interpreted to reflect renewals of the surface with liquid elements freshly brought to the interfacial zone by the agitator. The number of peaks on the curves (excluding valleys) was counted which was considered the number of renewals taking place, and s was then calculated. With the Beckman bulk oxygen probe, the mass transfer coefficient k_L was determined by using the following equation:

$$\bar{r} = \frac{dC}{dt} = k_L A (C^* - C) \qquad (5)$$

A in Equation (5) was the surface area of the absorbing liquid in tube F (Figure 2). With the special arrangements of the absorption cell described before, A was considered constant even as the agitator speed was varied and was calculated from the inside diameter (7/8 in.) of tube F. C^* is the dissolved oxygen concentration at the gas-liquid interface which is customarily assumed to be in equilibrium with oxygen in the gas phase in tube F. Linear plots were obtained between $s^{0.5}$ and k_L at different agitator speeds; a typical set of results is shown in Figure 4, with additional sets given in a thesis by D. D. Lee (1973). The linear relationship agrees well with that reported by Bungay, Huang, and Sanders

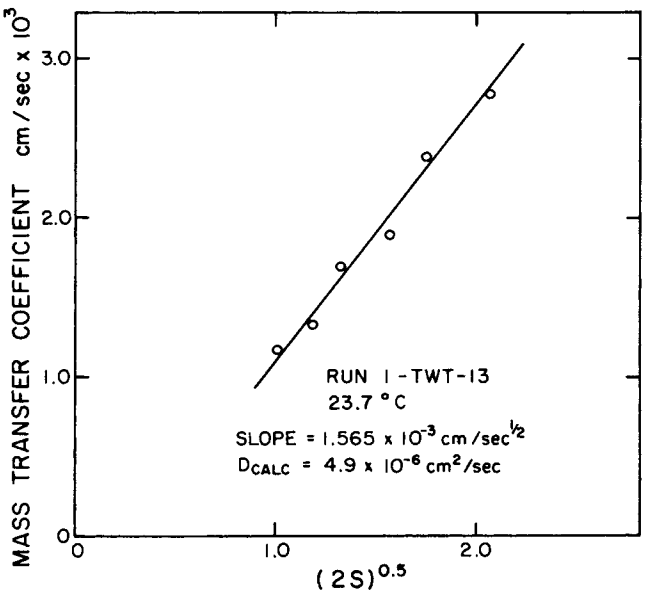


Fig. 4. Mass transfer coefficient vs. square root of surface renewal rate of Danckwerts.

(1973) and provides a direct experimental support to the Danckwerts theory which predicts Equation (4).

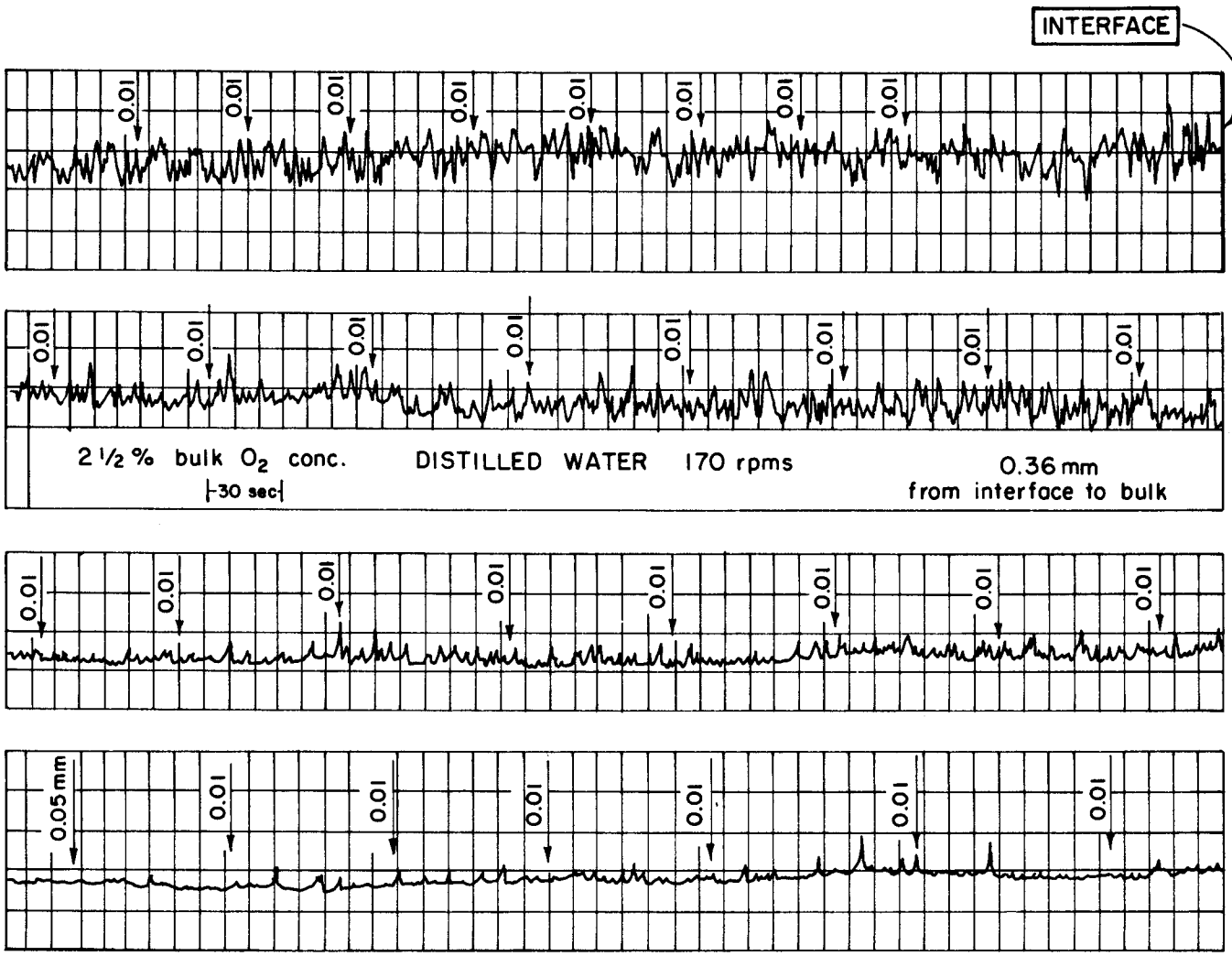


Fig. 5. Surface renewal at different distances from the interface into the liquid bulk.

RESULTS ON FLUCTUATION DECAY

An air-water pair was again used in the absorption cell. In this experiment, starting from the interface, the sensing tip was lowered by small increments towards the liquid bulk by carefully adjusting the micromanipulator. The recorder curve so obtained is shown in Figure 5, which is marked with arrows to show the discrete movements of the probe. Starting from the upper right-hand corner of Figure 5, as the microprobe moved downwards, the amplitude of the fluctuations on the recorder chart diminished. When the fluctuations completely died off, the sensing probe probably had reached the region which is normally considered the bulk of the liquid and where the concentration of the transferring solute (oxygen) should be uniform owing to agitation. The use of the microprobe thus has given a direct indication of the thickness of the penetration zone next to the interface.

RESULTS WITH SURFACTANTS ADDED

In another experiment, a crude enzyme (glucose oxidase) preparation which contains surface active proteins was dissolved in the water for the absorption study. The recorder chart is shown in Figure 6 starting from the upper right-hand corner. At the beginning, when the microprobe was very close to the air-liquid interface, little fluctuation in oxygen concentration was observed, which reflected the stagnant nature of a film due to the absorbed protein. This provides an experimental evidence indicating that the Danckwerts' type of surface renewal did not exist here because the liquid elements did not seem to be able to penetrate to the interface. As the sensing probe was lowered, the amplitude of the fluctuations increased, reflecting increased renewal by liquid elements from the liquid bulk. At a critical depth (0.24 mm in Figure 6), the oxygen concentration on the chart dropped abruptly and strong fluctuations started. The abrupt drop shows the piercing by the microprobe through the whole thickness of the stagnant film. As the sensing tip was lowered further, a similar decay in fluctuation amplitude was also observed as in the air-water system (Figure 5 with no protein added). Therefore, the experiment has shown the existence of two transferring zones near a gas-liquid interface: a stagnant film due to surface absorption of surfactants and a zone with ordinary kind of renewal and penetration by liquid elements from the liquid bulk. This picture agrees with an early postulate by Tsao (1972).

DISCUSSIONS

1. The use of microprobes should allow a direct experimental determination of the concentration profile of a transferring solute at a gas-liquid interface. This will require a proper damping of the recorder fluctuations; the short-time average of the sensor signals will give the local concentration profile. Future work in this area should be useful.

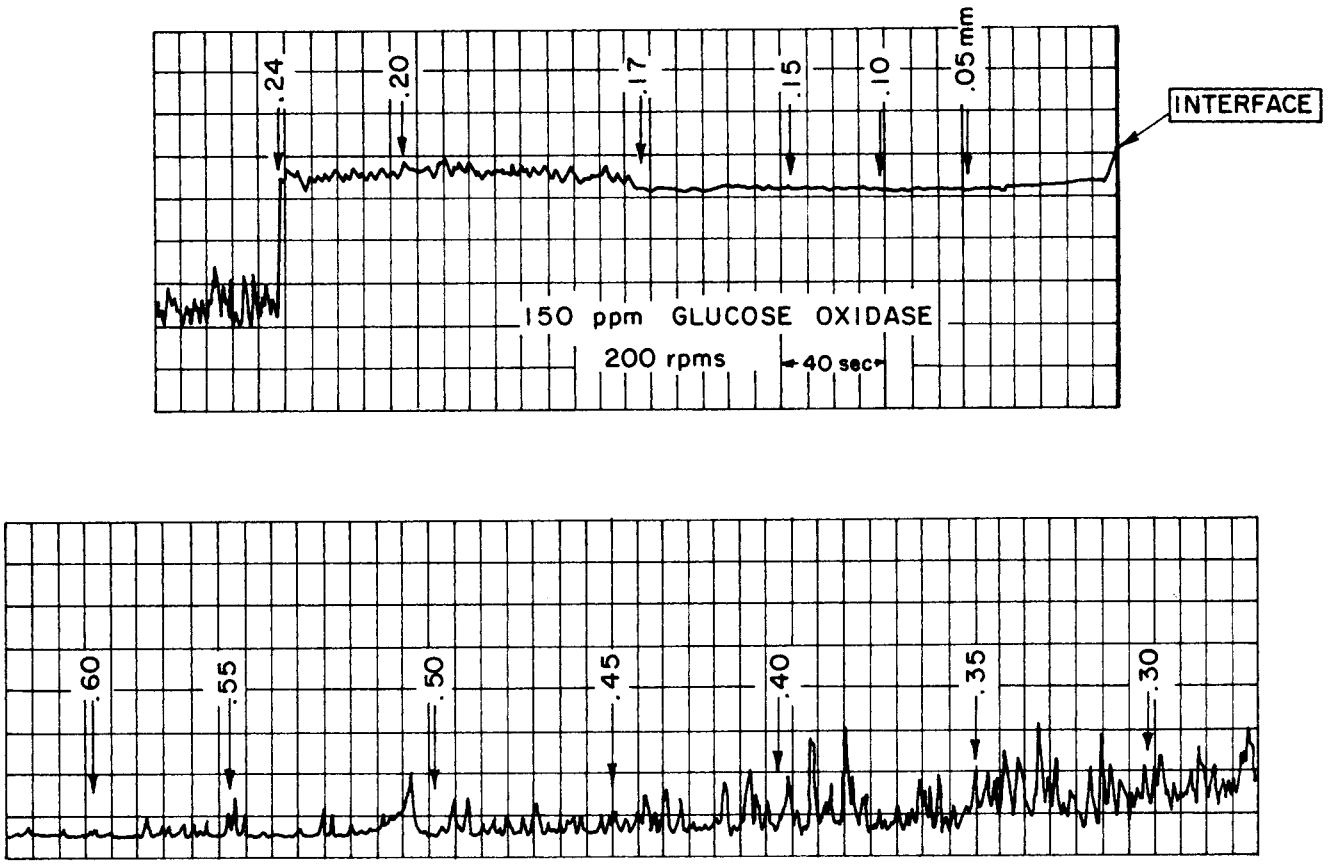


Fig. 6. The effect of added protein on surface renewal.

2. As the sensing tip is lowered towards the liquid bulk in an air-water pair, it would be interesting to know whether or not the frequency of renewal is changed. From the curve in Figure 5, the frequency seemed to remain more or less constant, but the amplitude changed drastically. Further analysis of the recorded curve may provide information for further refinement of the existing gas-liquid mass transfer theories.

3. Based upon his experimental data from carbon dioxide absorption into an aqueous buffer solution in the presence of an enzyme, carbonic anhydrase, Tsao (1972) derived two simultaneous partial differential equations, using the two-zone model in Figure 7. This model assumes a stagnant zone and also a renewal-penetration zone which is in essence a simplification of a more general model shown in Figure 1:

$$\frac{\partial C}{\partial t} = D_s \frac{\partial^2 C}{\partial x^2} - r(e_o, C) \quad 0 < x < h \tag{6}$$

$$\frac{\partial C}{\partial t} = D_L \frac{\partial^2 C}{\partial w^2} - r(e, C) \quad x > h \tag{7}$$

with boundary conditions

$$x = 0, \quad c = c^* \tag{8}$$

$$x = h, \quad c = c_h \tag{9}$$

$$t = 0, \quad x \geqq 0, \quad w \geqq 0, \quad c = 0 \tag{10}$$

$$t > 0, \quad w = 0, \quad c = c_h \tag{11}$$

$$t > 0, \quad w = \infty, \quad c = 0 \tag{12}$$

and

$$x = h, \quad D_s \left(\frac{\partial c}{\partial x}\right)_{x=h_-} = D_L \left(\frac{\partial c}{\partial x}\right)_{x=h_+} \tag{13}$$

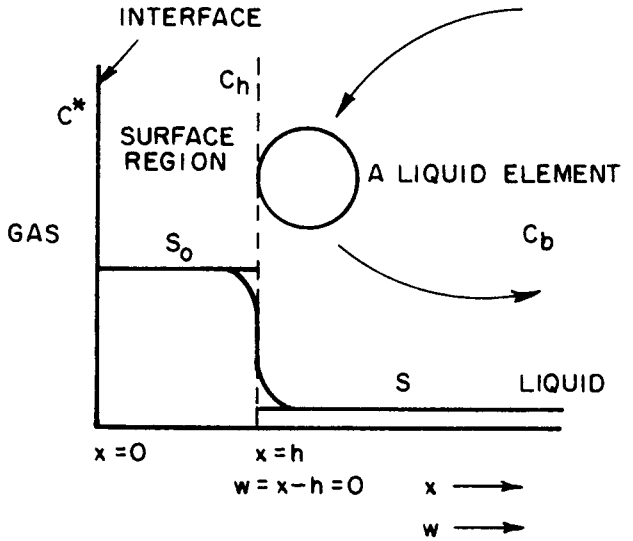


Fig. 7. A two-zone model of a gas-liquid interface in the presence of small particles.

Boundary conditions (8) through (12) are conventional. Equation (13) is a consequence of the solute balance for $x = h$.

Solutions of Equations (6) and (7), of course, will depend upon the specific expressions of $r(e_o, c)$ and $r(e, c)$ which are the consumption (or uptake) rates of the transferring solute by the enzyme molecules (or the microbiological cells in a fermentation system). Some special solutions of Equations (6) and (7) were given by Tsao (1972).

The results of this paper give a value for h. According to Figure 6, h is about 0.24 mm in the current system.

As mentioned above, with a proper damping of the signals, C_h can also be measured. This is the information needed in making numerical computations of oxygen transfer rates by using Equations (6) and (7).

ACKNOWLEDGMENT

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NOTATION

a = surface area of small particles (microbial cells) per unit volume of particles
A = gas-liquid interface per unit volume of liquid
C = concentration of the transferring solute
C_h = C at $x = h$
C^* = C at $x = 0$
D = solute diffusivity
D_s = D in the surface zone
D_L = D in the liquid bulk
e = enzyme concentration or microbial cell concentration
e_o = e in the surface zone
h = thickness of the surface zone
k_L = liquid side mass transfer coefficient
r = rate of interfacial mass transfer
$\bar{r}$ = time average of r
s = concentration of microbial cells and also Danckwerts surface renewal rate
R = rate of solute uptake by the microbial cells
t = time
x = distance from the interface
w = $w = x - h$

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Drug Permeation Through Human Skin: Theory and in Vitro Experimental Measurement

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The penetration of drugs and other micromolecules through intact human skin can be regarded as a process of dissolution and molecular diffusion through a composite, multilayer membrane, whose principal barrier to transport is localized within the stratum corneum. A mathematical model of the stratum corneum as a two-phase protein-lipid heterogeneous membrane (in which the lipid phase is continuous) correlates the permeability of the membrane to a specific penetrant with the water solubility of the penetrant and with its lipid-protein partition coefficient.

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